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A New *Hymenophyllum* Species in the Appalachians Represented by Independent Gametophyte Colonies

CATHERINE A. RAINE
Department of Cell and Structural Biology, University of Manchester, Manchester M13 9PL England

DONALD R. FARRAR
Department of Botany, Iowa State University, Ames, Iowa 50011

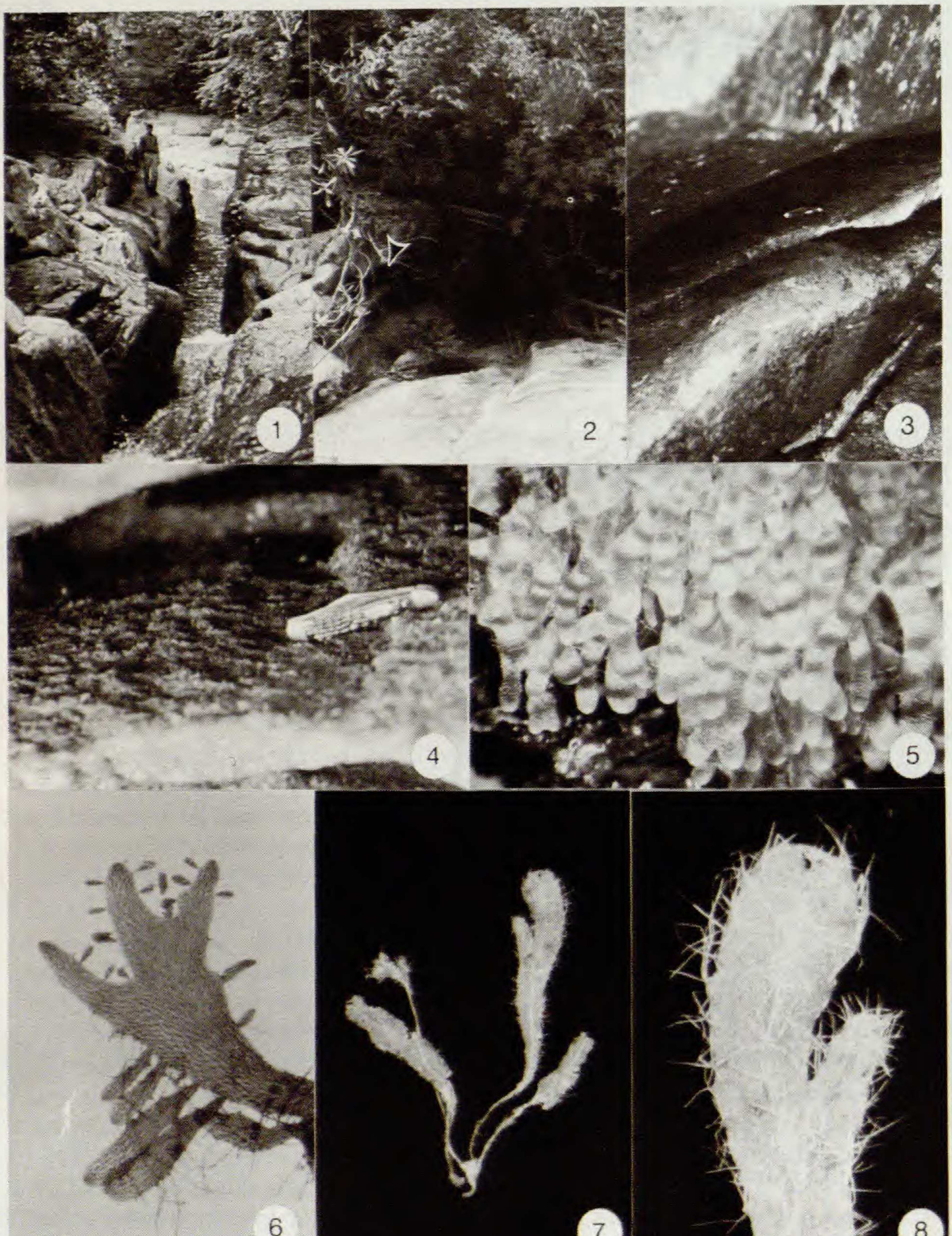
ELIZABETH SHEFFIELD
Department of Cell and Structural Biology, University of Manchester, Manchester M13 9PL England

In the United States, four genera of ferns are known to exist as independent gametophyte populations (Farrar, 1967). They are able to persist, in isolation from sporophytes of the species, by vegetative reproduction, including the production of gemmae. Their apparent inability to produce normal sporophytes has led to considerable difficulty in species identification.

The earliest vouchered collection of *Hymenophyllum* gametophytes in the southern Appalachian Mountains is one by Mary Taylor. She described the plants as growing "not far from one of the stations for *Hymenophyllum tunbrigense.*" The widespread occurrence of independent *Hymenophyllum* gametophytes in North and South Carolina was first reported by Farrar (1967). He, at that time, presumed the gametophytes (Figs. 1–6) to be "the same species as the sporophyte *Hymenophyllum tunbrigense* (L.) J. Sm. which grows in South Carolina." Subsequent publications either supported this possibility (Farrar, 1985) or remained uncommitted regarding their identity (Wagner et al., 1970). However, the rarity (Wagner et al., 1970) and relative sterility (Farrar, 1971) of *H. tunbrigense* sporophytes in the Appalachians, has always been difficult to reconcile with the frequent occurrence of independent gametophytes. Furthermore, Taylor described the gametophytes in her collection as "decidedly different from the prothallia that I think are those of *H. tunbrigense*" (pers. comm. to W. R. Maxon, 13 Oct., 1936).

There are several problems in determining whether these gametophytes are *H. tunbrigense* or not. Most studies of *Hymenophyllum* gametophytes (Atkinson, 1960; Goebel, 1888; 1905; Holloway, 1930; Stokey, 1940; 1948; Stone, 1965), have concentrated on development rather than on distinguishing features of the mature prothalli. Few studies have specifically described characters of *H. tunbrigense* gametophytes, and those that we have been able to find included no helpful figures (e.g. Janczewski & Rostafinski, 1875; Richards & Evans, 1972). Also, Farrar (1971) was unable to grow *H. tunbrigense* gametophytes in culture, and therefore did not obtain experimental data regarding gametophyte form and growth requirements and responses that would have been useful in this study.

The discovery of a single juvenile sporophyte of a second *Hymenophyllum* species in the United States (Wagner et al., 1970) further calls into question the



FIGS. 1–8. *Hymenophyllum* in North and South Carolina. FIG. 1. Typical site of independent *Hymenophyllum* gametophytes and *H. tunbrigense* sporophytes along the Eastatoe River in Pickens Co., S.C. FIG. 2. Typical habitat (arrowhead) of *Hymenophyllum* gametophytes in rock crevices along fast-flowing streams. FIGS. 3, 4. Close-up of *Hymenophyllum* gametophyte population in Fig. 2. FIG. 5. Gametophytes growing in culture (5X). FIG. 6. Gametophytes bearing marginal proliferations on older part of thallus and gemmae near the apices (6X). FIGS. 7, 8. Juvenile sporophyte of *Hymenophyllum* subgenus *Leptocionium* collected by Mary S. Taylor in Pickens Co., S.C. in 1936 (U.S. no. 1731687) (5X, 15X). Note stellate hairs characteristic of the subgenus.

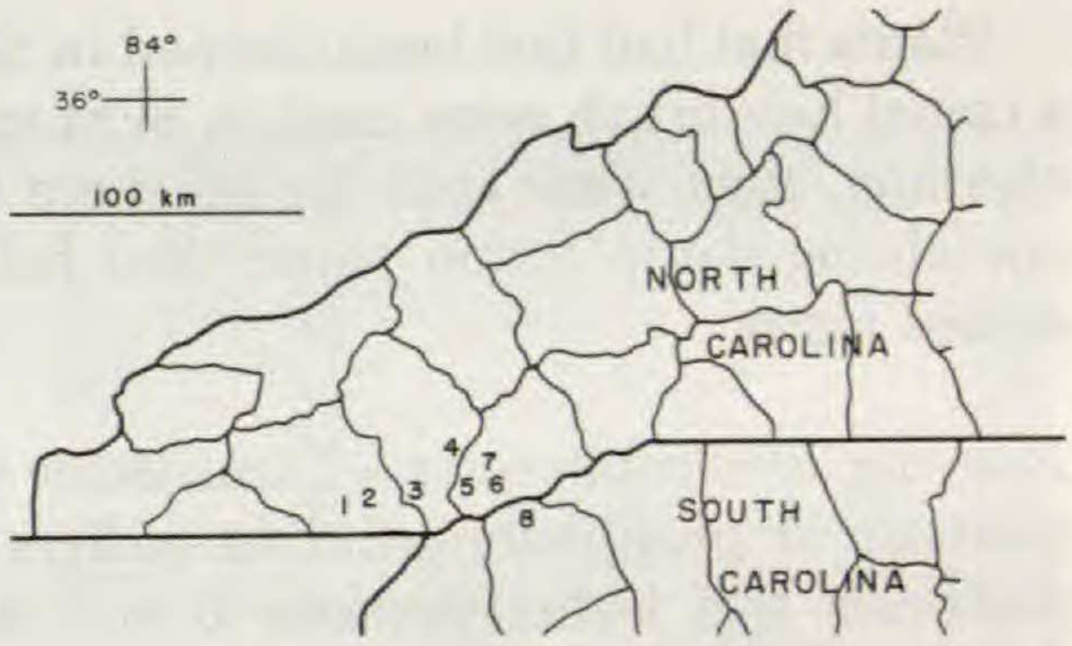


FIG. 9. Sites of live collections of the independent Appalachian *Hymenophyllum* gametophytes used in this study. 1 = Macon Co. Piney Knob Creek; 2 = Macon Co. Dry Falls, Cullasaja River; 3 = Jackson Co. Chattooga River at Norton Mill Branch; 4 = Jackson Co. Bonas Defeat, East Fork Tuckasegee River; 5 = Transylvania Co. Thompson River, many sites; 6 = Transylvania Co. Drift Falls, Horsepasture River; 7 = Transylvania Co. Schoolhouse Falls, Greenland Creek; 8 = Pickens Co. Eastatoe River, many sites.

identity of the independent gametophytes. A tiny sporophyte (Figs. 7, 8) found by Mary Taylor in 1936 was tentatively identified as *H. hirsutum* and has been included as such in subsequent literature (e.g. Proctor, 1985; Lellinger, 1987). However, on the basis of its laminar hairs, it keys to any one of three species (Morton, 1947): *H. trichophyllum, H. urbanii,* and most closely to *H. pulchellum.* The structure of its hairs is not precisely identical to any of these and is quite distinct from *H. hirsutum.*

Although a depressing number of papers maintain that subgeneric differences are not evident at a gametophytic level, some even referring specifically to *Hymenophyllum* in this context (e.g. Stokey, 1948; Stone, 1965), recent workers have documented taxonomically useful gametophytic characters. Techniques that have been employed for this purpose include scanning electron microscopy (SEM) (Sheffield & Farrar, 1988; Tigerschiold, 1989; Rumsey et al., 1990), and isozyme electrophoresis (e.g. Farrar, 1985), which is a sensitive indicator of genetic differences even when morphology is not. Our aim in this study was to establish the identity of the Appalachian species of *Hymenophyllum* gametophyte using morphological and electrophoretic characters.

MATERIALS AND METHODS

Independent *Hymenophyllum* gametophytes were collected during August 1989 from sites in North and South Carolina (Fig. 9). *H. tunbrigense* sporophytes were collected at the same time from sites in South Carolina. Gametophytes and sporophytes of *H. tunbrigense* were collected at Maentwrog, North Wales in October, 1989. Dr. J. T. Mickel kindly sent living sporophytic material of *H. pulchellum,* which was compared electrophoretically with independent gametophyte material.

Specimens were maintained either in the cold room or in culture, on *Sphagnum* peat, in the laboratory. The cultures were occasionally moistened with Bold's mineral nutrient solution (Bold, 1957). Permanent slides of all independent gametophyte samples were made by mounting specimens in Faure's gum chloral and by painting clear nail varnish around coverslip edges to prevent desiccation.

Plants that had first been cleaned in distilled water using gentle strokes with a camel hair brush were used in SEM and electrophoretic analysis. Following cleaning, they were kept for no more than two days in sealed petri dishes containing damp tissue paper. Just before use they were blotted to remove excess water.

Isozyme Electrophoresis.—Extractions were made using either the tris, tris-maleate, or phosphate grinding buffers of Soltis et al. (1983). Their staining protocols and buffer systems 6 and 8, or the morpholine (M) systems of Odrzykoski & Gottlieb (1984), were used to analyze aspartate amino transferase (AAT), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), malate dehydrogenase (MDH), shikimic dehydrogenase (SKDH), isocitrate dehydrogenase (IDH), peroxidase (PER), triose phosphate isomerase (TPI), 6 phosphogluconate dehydrogenase (6PGD) and fluorescent esterase (FE).

Scanning Electron Microscopy.—The morphologies of Welsh *H. tunbrigense* gametophytes and independent *Hymenophyllum* gametophytes from the Appalachians were investigated using a Cambridge S200 SEM fitted with a low temperature stage, using the methods of Sheffield & Farrar (1988).

Results

Isozyme Electrophoresis.—Not all the enzymes were well resolved in all specimens of independent gametophytes, but no variation was observed among nine samples from four sites. From this we conclude that all the North American independent gametophyte collections represent the same species. With the exception of one PGM band in some specimens of *H. tunbrigense,* the independent gametophytes shared no bands (of 19 total) with either *H. tunbrigense* or *H. pulchellum* (Fig. 10).

Morphological Features.—Many gametophytic features proved extremely variable and of 54 characters initially investigated, only nine seemed to vary interspecifically. Other features were either too variable (e.g. thallus length, rhizoid length and number, meristem width) or too conservative (e.g. rhizoid width and position) to be used. It was not possible to extensively investigate the morphology of antheridia and archegonia in the Appalachian gametophytes because they were very rare in our cultures. Table 1 lists features that distinguish the independent Appalachian gametophytes from those of *H. tunbrigense.*

The gemmae of the independent gametophytes are characteristic of *Hymenophyllum* subg. *Leptocionium,* the subgenus previously suggested for Taylor's sporophyte, as compared to those of subg. *Mecodium,* the other gemma-producing subgenus of tropical America. In the latter, basal cells on either side of the attachment cell become conspicuously swollen and generally protrude downward beyond the attachment cell. No similar growth occurs in gemmae of

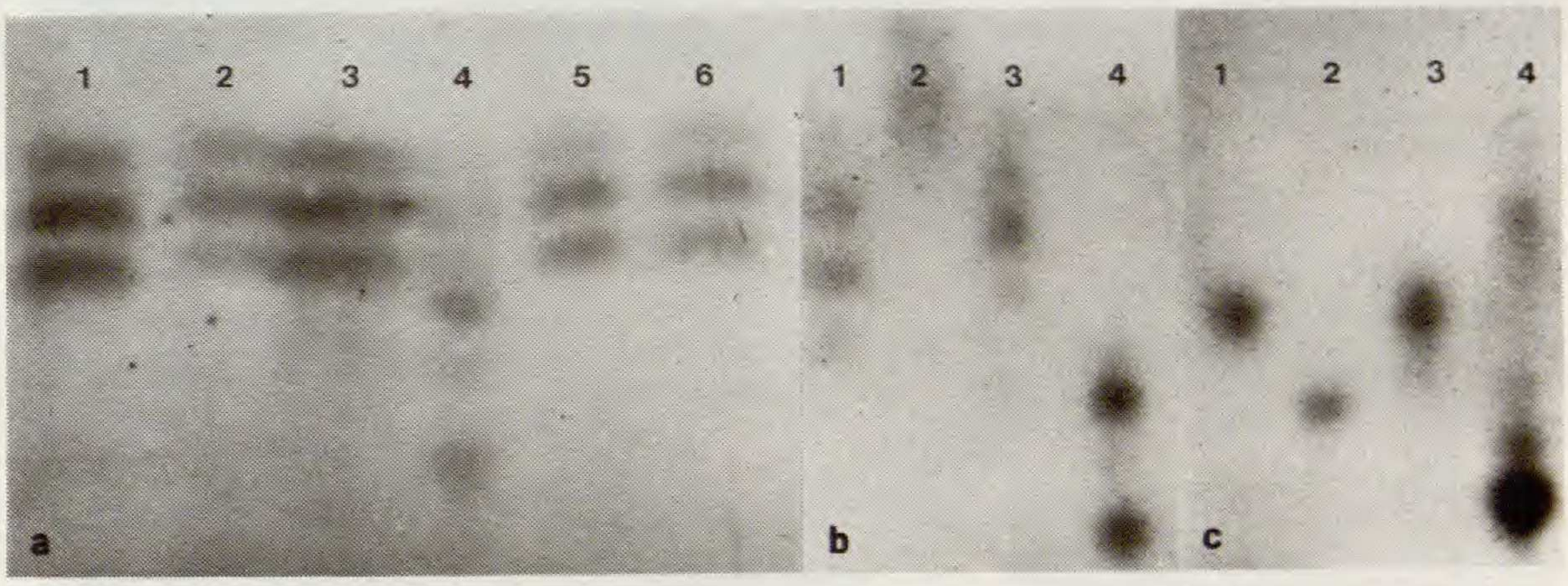


FIG. 10. Enzyme banding patterns in *Hymenophyllum*. a. MDH banding pattern of the independent Appalachian *Hymenophyllum* gametophyte (Lane 4) compared with both Welsh *H. tunbrigense* (Lanes 1 and 3 sporophytes, 5 and 6 gametophytes) and an *H. tunbrigense* sporophyte from the United States (Lane 2). b. MDH banding patterns. Lane 1, *H. tunbrigense* sporophyte; Lane 2, *H. pulchellum* sporophyte; Lane 3, *H. tunbrigense* sporophyte; Lane 4, Independent gametophyte. c. 6PGD banding patterns. Lanes as in b.

TABLE 1. Morphological characteristics of gametophytes of *Hymenophyllum tunbrigense* and the independent Appalachian *Hymenophyllum* gametophyte.

Hymenophyllum tunbrigense	Independent *Hymenophyllum*
Gemmae absent	Gemmae present
Margin entire, composed predominantly of straight-sided cells	Margin crenated, composed predominantly of cells with concave outer walls
Archegonia and antheridia common, often present on the same gametophyte	Archegonia and antheridia rare
Rosette growth habit	Sprawling growth habit
Branches always broad	Branches filamentous to broad
Proliferations few, always marginal	Proliferations abundant, arising at margins and centrally

subg. *Leptocionium* (Fig. 11) (Goebel, 1888; Stone, 1965; Farrar, unpublished observations on Hawaiian Hymenophyllaceae). Neither gemmae nor gemmifers were present in gametophytes of *H. tunbrigense* and have not been reported in this or other species of subgenus *Hymenophyllum*, the third subgenus of American *Hymenophyllum* (Janczewski & Rostafinski, 1875; Stone, 1965; Richards & Evans, 1972; Yoroi, 1972).

Crenate margins were a distinctive and constant feature observed in the independent gametophytes, although regenerated branches, or proliferations,

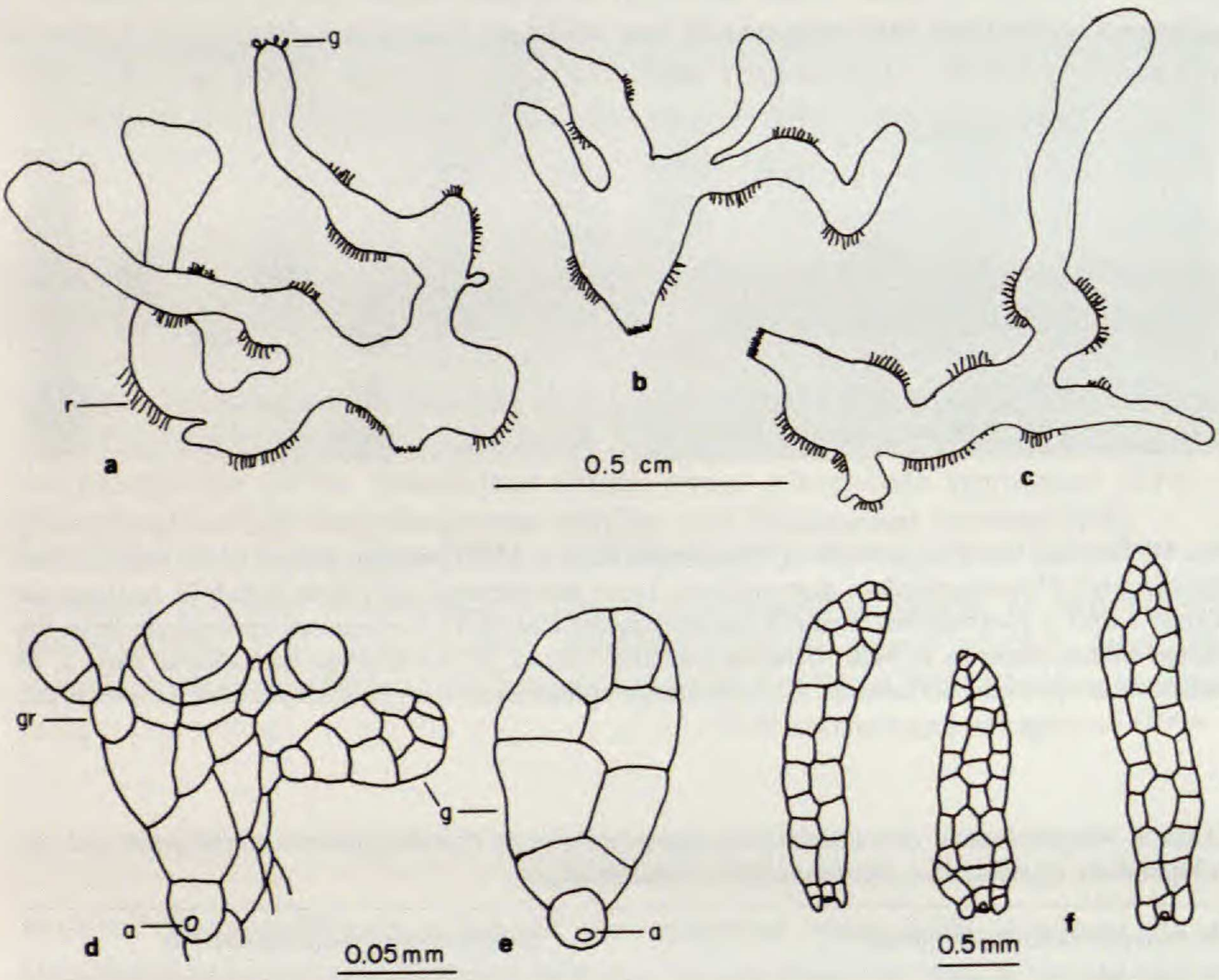
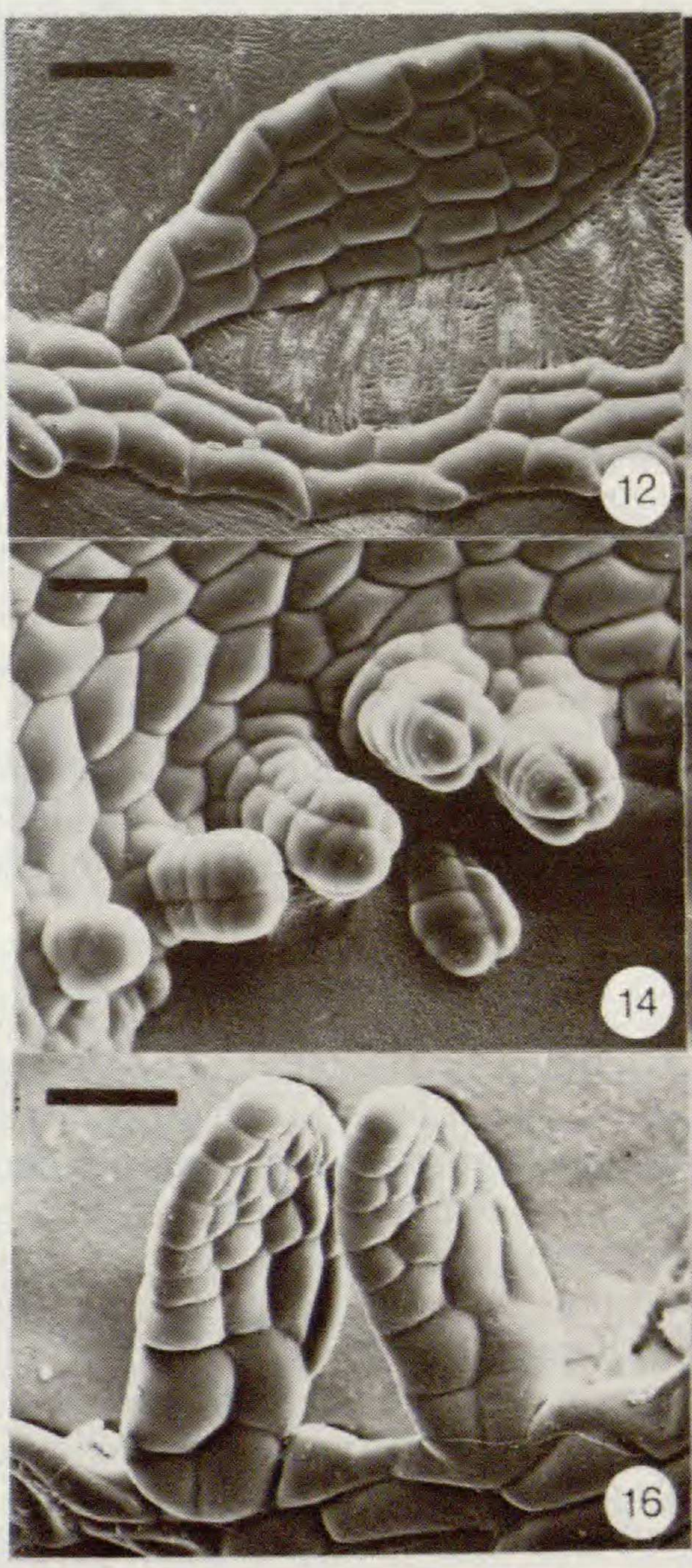
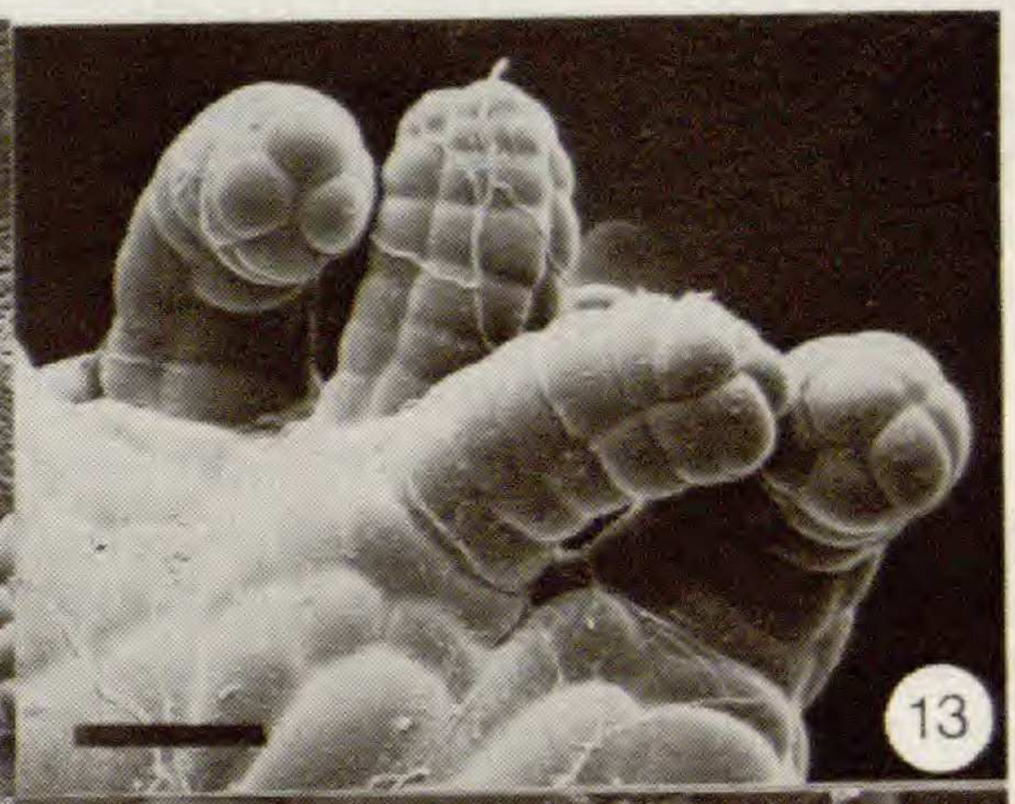


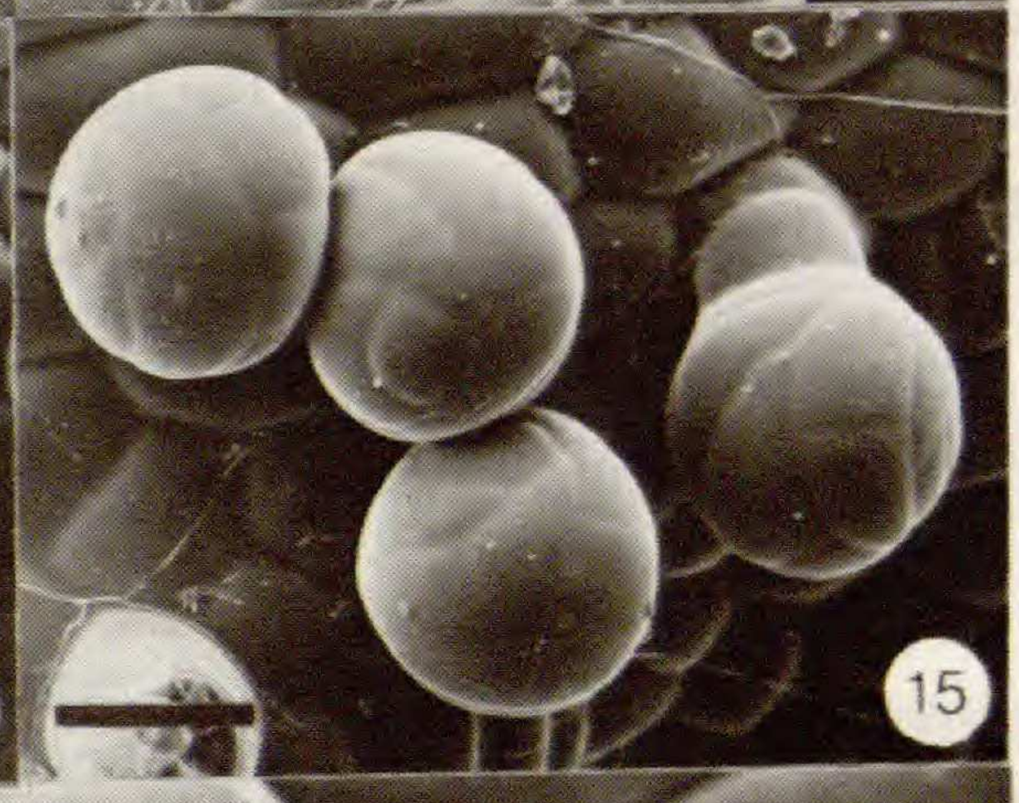
FIG. 11. Gametophytes and gemmae of the independent Appalachian *Hymenophyllum* gametophytes (subgenus *Leptocionium*) compared with gemmae of *H. wrightii* (subgenus Mecodium). a–e. Independent Appalachian *Hymenophyllum* gametophyte. a–c. Mature gametophytes. d. Gemma-producing apex. e. Mature dehisced gemmae. f. Mature dehisced gemmae of *H. wrightii*. Note protrusion of basal cells beyond attachment cell.

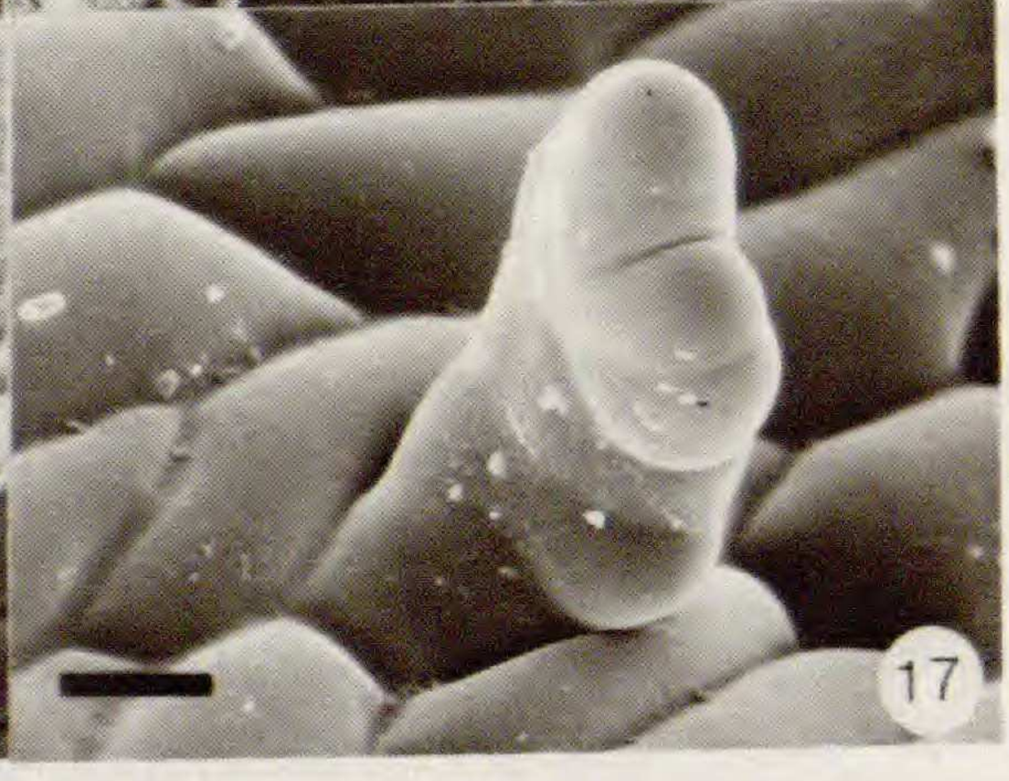
had less pronounced crenations (Fig. 12). Occasionally some of the marginal cells are sclerified. These features have not been specifically noted in other species.

Archegonia were present in three cultures of Appalachian gametophytes (Fig. 13), but no antheridia were found. Farrar (1971) found antheridia to be rare in field collected plants. Archegonia were abundant on *H. tunbrigense* gametophytes (Fig. 14) and were often associated with antheridia (Fig. 15). Mature *H. tunbrigense* gametophytes resembled the classic rosette form described by Holloway (1930) and Stokey (1940), rather than the sprawling ribbon form of the independent gametophytes. The Appalachian gametophytes fail to attain rosette form in part because they frequently produce branches with narrow bases that are easily detached (Fig. 16). These proliferations act as a further vegetative means of reproducing the colony. Proliferations from the center of the thallus, which developed after a year in culture, have not been noted in *Hymenophyllum* gametophytes in previous studies (Fig. 17).









FIGS. 12–17. Gametophytes of *Hymenophyllum*. FIG. 12. Independent gametophyte with crenated margins and with a proliferation bearing less pronounced crenations. Bar = 100 μm. FIG. 13. Archegonia of the independent gametophyte. Note pronounced curvature in comparison with those of *H. tunbrigense*. Bar = 50 μm. FIG. 14. Archegonia of *H. tunbrigense*. Bar = 50 μm. FIG. 15. Antheridia of *H. tunbrigense*. Bar = 50 μm. FIG. 16. Marginal proliferations on an independent gametophyte. Bar = 50 μm. FIG. 17. Young central proliferation illustrating its development from the middle of the gametophyte. Bar = 25 μm.

DISCUSSION AND CONCLUSIONS

Electrophoretic and morphological analyses indicate that the independent Appalachian *Hymenophyllum* gametophytes cannot be *H. tunbrigense*. Enzyme

banding patterns of *H. pulchellum* sporophytes were likewise sufficiently distinct from those of the independent gametophytes to dismiss the possibility that they could be conspecific. Although a wide range of morphologies results from the plants' responses to differing environments superimposed on the innate variation of gametophytes, a number of morphological features reliably distinguish the independent gametophytes from those of *H. tunbrigense.*

On the basis of current evidence, the independent *Hymenophyllum* gametophytes cannot be identified as any named species. As with the independent Appalachian *Vittaria* gametophytes (Farrar, 1990), they possibly are an ancient species long isolated in the eastern United States and distinct from any existing tropical species. We propose *H. tayloriae* as a suitable name, in honor of Mary Taylor, the discoverer of both *Hymenophyllum* species presently known from South Carolina (Taylor, 1938; Wagner et al., 1970).

Hymenophyllum tayloriae Farrar and Raine, sp. nov. (Figs. 4–6, 11a–e, 12–13, 16–17.)

Plantae in statu gametophytico tantum existens; thalli ramosi ecostati tenues, cellularum in strato crassitie unae cellulae compositi; rhizoidea ad marginem limitatus; meristemata ramorum rotundata gemmipara; gemmae spathulatae, 5–8 cellulas longae, 2–4 cellulas latae.

Sporophyte lacking. Gametophyte yellow-green, epipetric or occasionally epiphytic on roots, perennial and clone-forming by vegetative reproduction. Mature plants composed of an irregularly branched, ribbon-like thallus one cell in thickness. Growth indeterminate by marginal meristems at the rounded ends of branches. Branches arising by division of terminal meristems and by proliferations from marginal and occasionally from medial cells of older portions of the thallus. Branches 0.1–1.0 mm. wide and up to 1.0 cm long. Margins of the thallus often crenate by curvature of the cell walls. Marginal cells occasionally sclerified. Rhizoids short, brown, emanating only from marginal cells of the thallus. Aerial branches frequently terminating in production of gemmae. Gemmae composed of spathulate plates of cells 0.1–0.2 mm long, 5–8 cells long and 2–4 cells wide, each attached to the thallus by way of an orbicular gemmifer cell that remains attached to the thallus after the gemma is shed. Archegonia clustered on cushions along the margins of large thalli. Antheridia on thallus margins.

Type: U.S.A.: South Carolina: Pickens Co., Eastatoe River below junction with Rocky Bottom Creek, under rock outcrops along river, 22 June 1970, *Farrar 1312b* (holotype ISC; isotypes MICH, MO, NC, NY, UC, US).

Representative Specimens: **U.S.A.: North Carolina: Jackson Co.,** Bonas Defeat, moist ledge by waterfall, 23 Aug 1951, *Anderson 10593* (ISC); Chattooga River at junction with Norton Mill Creek, under rock ledges, 6 Aug 1966, *Farrar 1121* (ISC, MICH, MO, NC, NY, UC, US); Wolf Creek Falls, *Pittillo & Wolfe s.n.* (ISC); **Macon Co.,** Falls on Piney Knob Creek, in crevices east side of falls, 2 Aug 1966, *Farrar 1111* (ISC, MICH, MO, NC, NY, UC, US); Falls on Piney Knob Creek, in moss mats on boulders in stream below falls, 2 Aug 1966, *Farrar 1112* (ISC, MICH, NY, UC, US); Dry Falls, under cliffs on east side of falls, 23 Aug 1989, *Farrar 89-8-23-1* (ISC, US); **Transylvania Co.,** Drift Falls on Horsepasture River, in crevices in rock outcrops on west side of river below the falls, 21 Aug 1989, *Farrar 89-8-21-7* (ISC, NY, US); Schoolhouse Falls, on soil and root masses under cliffs west of falls,

20 Aug 1989, *Farrar 89-8-20-8* (ISC, MICH, MO, NC, NY, UC, US); Thompson River Falls, under cliffs below falls, 26 July 1966, *Farrar 1092* (ISC, MICH, NY, UC, US); **South Carolina: Pickens Co.,** Eastatoe River, moist shaded rock in deep ravine, 19 April 1936, *Taylor s.n.* (US #1731687); Eastatoe River, cliffs along river at lower end of gorge, 24 Aug 1989, *Farrar 89-8-24-1* (ISC, MICH, MO, NC, NY, UC, US); Eastatoe River, cliffs along river between upper narrows and Rocky Bottom Creek, with *H. tunbrigense,* 24 Aug 1989, *Farrar 89-8-24-18* (ISC, MICH, NY, UC, US); Rocky Bottom Creek near junction with Eastatoe River, on north-facing cliffs in narrow gorge, 24 Aug 1989, *Farrar 89-8-24-22* (ISC, MICH, MO, NC, NY, UC, US).

Mrs. Taylor's 1936 collection containing the single, tiny, juvenile sporophyte also contained gametophytes of *H. tayloriae,* although no gametophytes are organically connected to the sporophyte. The most diagnostic character of the sporophyte is the stalked stellate hairs attached to the margins and midrib but not to the lamina of the leaf. Such hairs and their placement are characteristic of subg. *Sphaerocionium,* section *Sphaerocionium,* subsection *Ciliata* of Morton (1947, 1968). *Leptocionium* is now considered to be the appropriate name for this subgenus.

A gametophytic character of *H. tayloriae* allies it also with subg. *Leptocionium* and thus with Mrs. Taylor's juvenile sporophyte. The unmodified basal cells of the gemmae of *H. tayloriae* are similar to those of other species of subg. *Leptocionium* and unlike those of subg. *Mecodium* (or those of subg. *Hymenophyllum* which apparently do not produce gemmae). On the basis of co-occurrence and this morphological evidence, we consider Mrs. Taylor's juvenile sporophyte to be probably conspecific with *H. tayloriae.*

H. tayloriae is distinguished from the independent gametophytes of *Vittaria appalachiana* Farrar and Mickel by its 2-dimensional spathulate gemmae (those of *V. appalachiana* are uniseriate), rhizoid attachment only to marginal cells, yellow-green color, and glossy texture. Thalloid liverworts of similar size are generally more than one cell thick or have a distinct midrib, have notched apical meristems, and do not produce spathulate gemmae.

H. tayloriae is found in moist, deeply shaded microhabitats in crevices of noncalcareous rock outcrops and under overhanging rocks along fast-flowing streams and waterfalls. In these habitats, plants grow attached to bare rock, thin soil, or root masses. Occasionally they are found among mosses on boulders below waterfalls. The species is known from gorges of both north- and south-flowing rivers of the southern Blue Ridge escarpment in Pickens County, South Carolina and Macon, Jackson, and Transylvania counties in North Carolina.

The success of the Hymenophyllaceae has been ascribed in part to the ability of their gametophytes to regenerate from a few green cells and their capacity for gemma production (Stone, 1965). These means of vegetative reproduction and colony dispersal are exploited to the full by *H. tayloriae.* Rapid proliferation by branching as well as by gemmae allows *H. tayloriae* to exist as a distinct species in isolation from a sporophyte generation.

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